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Case Report

A case of endometrial cancer in the context of a *BRCA2* mutation and double heterozygosity for Lynch syndrome

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Introduction

Lynch syndrome, also called hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant cancer susceptibility syndrome caused by germline mutations in DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, and less frequently *MSH6* and *PMS2*. MMR mutation carriers are predisposed primarily to colorectal cancer and endometrial cancer, with an increased frequency of stomach, ovary, pancreas, upper urinary tract, brain, small bowel, and skin consistently reported. This hereditary syndrome accounts for approximately 2–3% of colorectal cancers and 1–4% of endometrial cancers in the United States (Lynch and de la Chapelle, 2003). Depending on the MMR gene involved, women with Lynch syndrome can have up to an 80% lifetime risk of developing colorectal cancer, and a 20–60% risk of endometrial cancer.

Germline mutations in *BRCA1* or *BRCA2* (BRCA1/2) cause hereditary breast ovarian cancer syndrome. Female carriers of BRCA1/2 mutations have excessive risks for both breast and ovarian cancer, with lifetime breast cancer estimates ranging from 45% to 84%, and lifetime ovarian cancer estimates ranging from 11% to 62%, depending upon the population studied. BRCA1/2 kindreds are also noted to have an increased frequency of prostate cancer, and in *BRCA2* kindreds, increased frequencies of pancreatic cancer and melanoma are observed. The frequency of *BRCA1* or *BRCA2* mutations in the general population is estimated to be 1 in 300 to 1 in 800, respectively (King et al., 2003).

While there are kindreds with more than one cancer susceptibility syndrome and/or mutation reported in the literature (Thiffault et al., 2004; Smith et al., 2008), they are not often encountered in routine clinical settings. We report on a young woman who presented to our institution for prophylactic bilateral salpingo-oophorectomy (BSO) due to previously identified BRCA2 mutation, (i.e. 5946delT). An early endometrial cancer was identified. While endometrial cancer diagnosed under the age of 50 is not included in the Revised Bethesda Guideline, evidence suggests that these individuals should be evaluated for Lynch syndrome (Resnick et al., 2009). The patient presented was diagnosed with endometrial cancer at the age of 41 and genetic testing revealed triple heterozygosity for *BRCA2*, *MLH1* and *MSH6* mutations.

Clinical history

A 41-year-old G4P4 patient presented to our institution for prophylactic bilateral salpingo-oophorectomy due to a known BRCA2 mutation, which was also documented in her mother and consistent with several cases of ovarian cancer in this lineage. She had been followed with alternating serum CA125 and transvaginal ultrasound every 6 months, and annual mammogram and MRI of breast were negative. She then reported abnormal uterine bleeding. Dilation and curettage of the endometrium revealed complex endometrial hyperplasia with atypia. The patient underwent hysterectomy with bilateral salpingo-oophorectomy. Paternal family history at this time was notable only for an aunt with colon and stomach cancer in her 60's.

Histology and immunohistchemistry

The hysterectomy and bilateral salpingoophorectomy specimen was received in the pathology laboratory. The uterine fundus was sectioned and entirely submitted for histologic examination. The sections showed multiple residual foci of complex atypical hyperplasia and several scattered foci of well to moderately differentiated endometrioid adenocarcinoma (FIGO II) with superficial myometrial invasion (Fig. 1A–B). The neoplastic cells invaded the myometrium to a depth of 0.3 cm where the myometrium measured 2.0 cm in thickness. Scattered lymphocytic infiltration was identified at the margins of the tumor (40 tumor-infiltrating lymphocytes/10 high power fields) (Fig. 1C). Immunohistochemical stains for p53 were performed on several sections and demonstrated no evidence of papillary serous carcinoma component (Fig. 1D).

Microsatellite analysis by PCR

Normal and tumor tissues were microdissected and DNA was extracted separately from the different areas. PCR amplification was performed for the microsatellite loci including BAT25, BAT26, D2S123, D5S346, and D17S250, as recommended by the revised guidelines issued by the International Collaborative Group on

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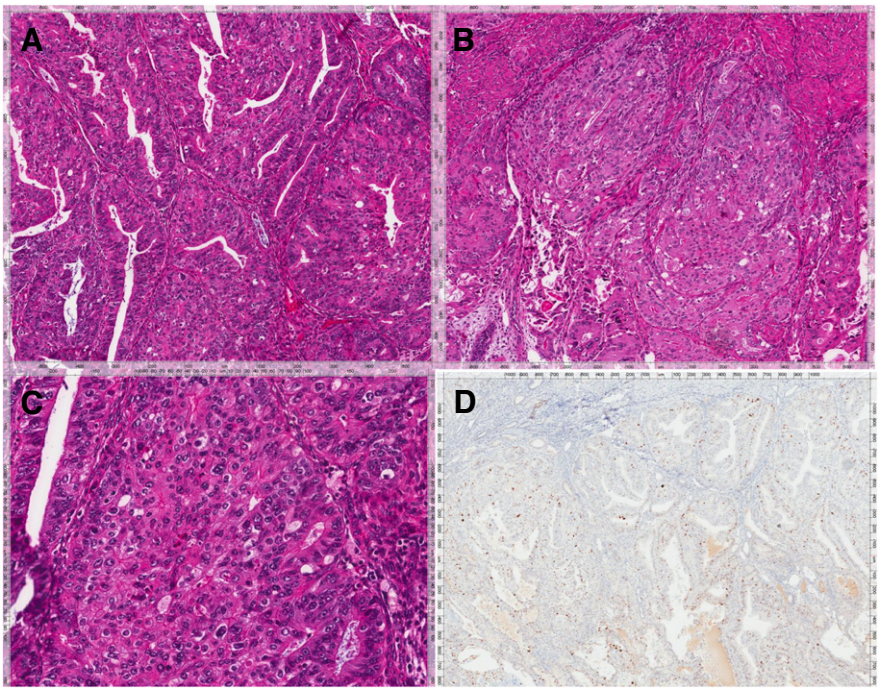

Fig. 1. Histology of the endometrial cancer. A. Moderately differentiated endometrioid adenocarcinoma. B. Superficial myometrial invasion of the tumor. C. Scattered lymphocytic infiltration into the tumor (at least 40 lymphocyte/10 high power field). D. Immunohistochemical staining of p53 shows negative staining. (A–C. Hematoxylin–eosin stain, 100×. D. Immunohistochemical stain of p53, 100×).

HNPCC and the National Cancer Institute. Tumor cells demonstrated microsatellite instability (MSI) in foci of BAT25, BAT26, and D5S346, suspicious for MSI in focus of D2S123, and microsatellite stable in D17S250 (Fig. 2). MSI in at least 2 out of 5 microsatellite loci is classified as MSI-high (MSI-H). So a DNA mismatch repair deficiency is present in this endometrioid carcinoma.

Immunohistochemistry (IHC) of mismatch repair (MMR) proteins

IHC of MMR proteins including MLH1, PMS2, MSH2, and MSH6 is based on determining complete loss or significantly attenuated nuclear staining for these proteins in the tumor compared with intact staining in adjacent normal tissue and inflammatory cells. This patient's tumor showed loss of MLH1 and PMS2 immunostaining and weak expression of MSH6 (Fig. 3). This patient must have a primary expression loss or abnormality of the hMLH1 protein, which causes advanced degradation of the normally expressed hPMS2 protein due to inability to form the hMutLa heterodimer. This profile is consistent with microsatellite instability.

Sequencing of MMR genes

The patient was referred for genetic counseling and germline Lynch syndrome testing based on her early onset endometrial cancer

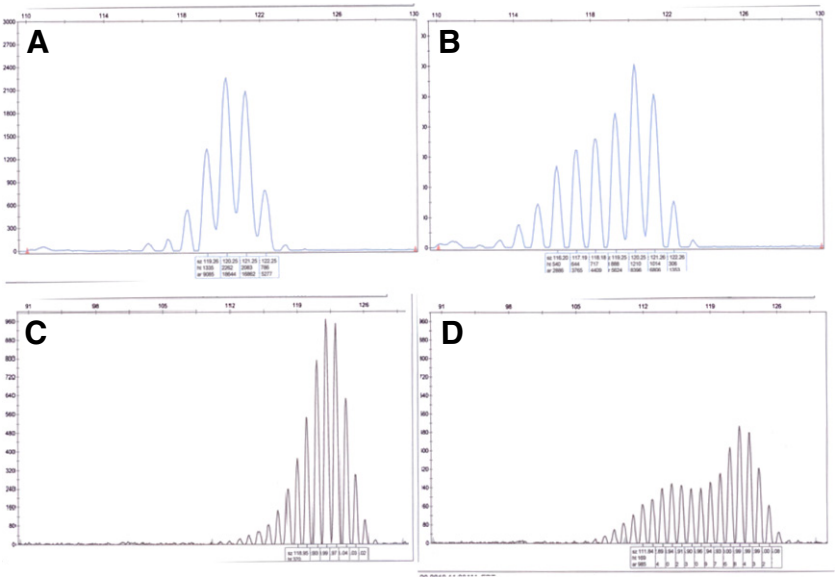

Fig. 2. Microsatellite instability by PCR. A. BAT25 in normal control. B. BAT25 with microsatellite instability in the patient. C. BAT 26 in normal control. D. BAT26 microinstability in the patient.

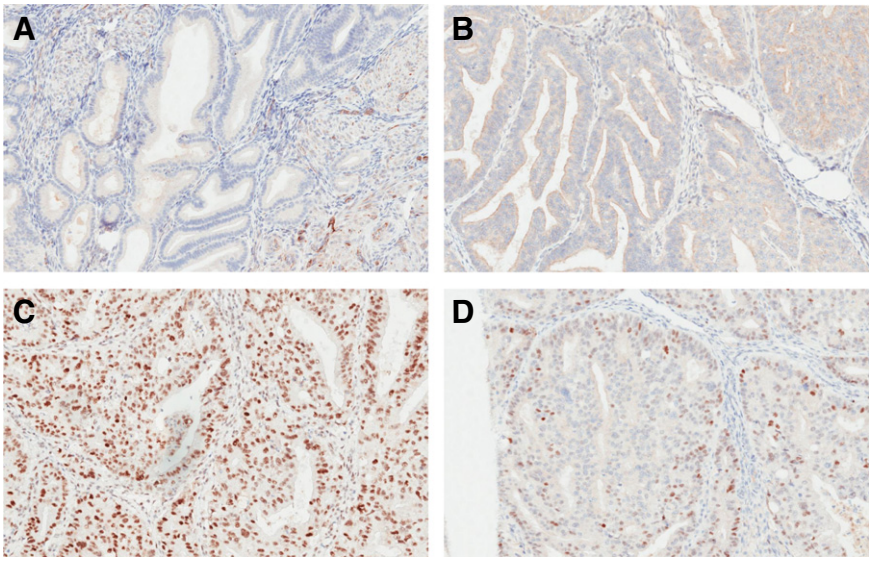

Fig. 3. Immunohistochemistry staining of mismatch repair proteins. A. Loss of MLH1 expression (200×). B. Loss of PMS2 expression (200×). C. Expression of MSH2 (200×). D. Weak expression of MSH6 (200×).

with an MSI-H phenotype. IHC of MMR proteins were not available at the time of genetic counseling, nor was precise information regarding a Lynch syndrome workup in her paternal lineage. Therefore, concurrent analysis of *MLH1*, *MSH2* and *MSH6* was requested on a peripheral blood sample. These studies revealed two deleterious MMR mutations: germline MLH1 mutation R226L (677 G > T), consisting of a nucleotide substitution immediately adjacent to intron 8; and germline MSH6 mutation 3959del4, resulting in premature truncation of the MSH6 protein at amino acid position 1325. While the patient's *MLH1* mutation carrier status was consistent with her tumor IHC profile and her aunt and cousin have only MLH1 mutation, the *MSH6* mutation was not expected. Because of the unusual nature of this case, a second sample was obtained for confirmatory germline studies, which again confirmed the presence of the *MLH1* and *MSH6* mutations.

Genetic counseling implications

The patient was followed up with colonoscopy, esophagogastroduodenoscopy and endoscopic retrograde cholangiopancreatogram (ERCP) to rule out any precancerous change or cancer in the gastrointestinal and hepatobilliary systems. No changes were seen in the esophagus, stomach, duodenum, pancreas, and small or large bowels.

Discussion

We reported a case of endometrial cancer in a young patient with both HNPCC and BRCA2 mutations. The tumor has the histologic features of endometrioid adenocarcinoma with tumor-infiltrating lymphocytes. The tumor infiltration lymphocytes are commonly seen in MSI related cancer, including endometrial cancer. The tumor also has positive MSI by PCR, loss of MLH1 and PMS2 by IHC, and mutations of MSH1 and MSH6 gene by genomic sequencing.

While it is clear that the patient inherited her *BRCA2* mutation from her mother, and it is presumed that she inherited her *MLH1* mutation from her father, the origin of her *MSH6* mutation is not clear based on pedigree analysis alone. *MSH6* carriers are at increased risk for gynecologic cancers and males have a lower colon cancer risk than *MLH1* or *MSH2* carriers. For this reason, the *MSH6* mutation could be "hiding" on either side of the family; the possibility of a *de novo* mutation also remains. Attempts to test parents have thus far been unsuccessful.

In the previous reported single kindred with both MSH2 and BRCA2 mutation, there are only two cases with double heterozygotes. The double heterozygotes in this kindred do not appear to have an earlier age of onset than carriers of a single mutation, and the cancers appear to have independent genetic aetiologies (Thiffault et al., 2004). Our patient has both BRCA2 and MMR (MSH1 and MSH6) mutations. BRCA2 mutation has an increased risk of serious papillary carcinoma involving ovaries and fallopian tubes. The endometrial carcinomas in BRCA2 positive patients are more likely to be serous type. The endometrioid cancer in our patient is more correlated with MLH1 and MSH6 mutation.

A primary expression loss or abnormality of the MLH1 protein will cause advanced degradation of the normally expressed PMS2 protein due to inability to form the hMutLa heterodimer (Karamurzin and Rutgers, 2009). Cases with primary mutation of MSH2 may show weak or negative immunohistochemical staining for both MSH2 and MSH6 due to the same mechanism of degradation of the MSH6 protein due to lack of stabilization of the hMutSa heterodimer. The primary mutation of PMS2 will result in isolated loss of PMS2. Similarly, the mutation of MSH6 will result in isolated loss of MSH6 (Karamurzin and Rutgers, 2009). Our patient had MLH1 mutation and showed loss of expression of MLH1 and PMS2.

Although the patient had MSH6 gene mutation, the IHC show weak expression of MSH6. A prior study investigating mutations and IHC findings in 25 index patients and 8 relatives with MSH6 variant was significant for deletion of 5 different truncating mutations and 10 variants of missense mutation in MSH6. Twelve of 18 tumors of truncating-mutation carriers and 3 of 17 tumors of missense-mutation carriers showed loss of MSH6 staining. Most missense mutation carriers still show MSH6 expression by immunostaining (Berends et al., 2002). In our case there was a truncating mutation at the end of the MSH6 protein, likely sufficient to produce protein that could be detected by IHC but was functionally aberrant. MSH6 mutation is usually inherited from parents rather than acquired after birth. The patient's aunt and cousin have only MLH1 mutation but no MSH6 mutation. So the mother's status of MSH6 should be further investigated.

Our case also raises questions about the ideal molecular workup for Lynch syndrome. Had we used an IHC guided approach, the MSH6 mutation would have been missed. Had we used the commonly accepted "test for MLH1 and MSH2, with reflex MSH6 testing only if the first two are negative" algorithm, the MSH6 mutation would have been missed. While double heterozygosity for Lynch syndrome might not affect the patient's cancer risk assessment and surveillance, it will

certainly affect the single site testing for her children. Aside from a 50/50 risk for hereditary breast ovarian cancer syndrome, each child has a 75% chance of having at least one Lynch syndrome causing mutation.

We are the first group to report concurrent MLH1 and MSH6 mutation in Lynch syndrome. However, more cases may have been missed by following current IHC guided approach or by MSH6 reflex test algorithm. MSH6 staining could still be positive with MSH6 missense mutation or terminal truncating mutation. We would recommend including MSH6 IHC in the initial screening test. When IHC results are equivocal, sequencing of MMR genes is the gold standard.

Different groups of investigators have reported that the risk of endometrial cancer is significantly lower in families with MLH1 mutation, but much higher in families with only MSH6 mutations, which indicates that the tumorigenic pathways between endometrial and colorectal cancers may be different. The TGFBR2(A)10 intragenic repeat showed instability in carcinomas of the rectum and urothelium, although it was not altered among the endometrial and ovarian tumors (Zaanan et al., 2011; Yamamoto et al., 2001). MSH6 inactivation may select for a different tumor suppressor gene.

Defects in MMR result in the accumulation of somatic mutations in genes containing microsatellite repeated sequences. Accumulation of such mutations is considered as the major molecular mechanism driving MMR-deficient cells oncogenic transformation. Several target genes involved in key cellular functions such as DNA damage signalling and repair, apoptosis, signal transduction and transcription regulation are suspected to play a critical role in tumour initiation and/or progression (Zaanan et al., 2011). Acquired BRCA2 gene mutation has been reported in a patient with widespread MSI (Yamamoto et al., 2001).

Patients with BRCA 1 or BRCA 2 mutation usually undergo prophylactic mastectomy and oophorectomy to prevent cancer. A recent study in 2011 investigates the cost-effectiveness of prophylactic surgery versus surveillance in women with Lynch syndrome and concluded that risk-reducing surgery is the least expensive option (Yang et al., 2011). More studies are suggesting that patients with germline mutations in DNA mismatch repair genes should receive counseling about colectomy, and if women, prophylactic hysterectomy and bilateral oophorectomy (Yang et al., 2011).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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